REMARKS

Claims 24-37 are presently pending and under consideration in this application. Claim 35 has been amended. Claim 37 is cancelled herewith because it was inadvertently miss-numbered as a second claim 36. Former claim 37 miss-numbered as a second claim 36 has been added as new claim 38. Support for new claims can be found throughout the instant Application, and particularly on pages 2 and 3 as well as in claims 1-22 as originally filed. No amendment should be construed as acquiescence in any ground of rejection.

35 USC § 102, Second Paragraph

Claims 28 and 29 are newly rejected under 35 U.S.C. § 112, second paragraph as allegedly being indefinite. The Examiner has asserted that Claim 28 drawn to a "knock out" is indefinite when it depends from claim 26 that is directed to a "vector." According to the Examiner, Claim 29 is included in this rejection because it depends from claim 28.

To clarify the invention, Applicant has amended claim 28 to depend from claim 24. Claim 29 continues to depend from claim 28. Applicant requests withdrawal of the rejection and reconsideration of the amended claims.

35 USC § 102, First Paragraph, Anticipation

Claims 35-37 have been newly rejected under 35 U.S.C. § 102(b) as being anticipated by the teachings of Chattopadhyay *et al.* (2000, Journal of Bacteriology, 182:6418-6423) for reasons

of record, April 10, 2007; January 4, 2008; and August 12, 2008, and March 19, 2009. The Examiner has further asserted that arguments filed March 19, 2009 to overcome this rejection have been fully considered, and in the Examiner's opinion, are not persuasive as to claims 35-37.

This rejection is respectfully traversed. In the instant amendment, claim 35 has been amended in claim 35 line 2, to remove reference to endogenous genes in causing the initial perturbation of the yeast cells. It remains the case that in response to an initial expression of a heterologous gene in the yeast, other endogenous genes may be downregulated or upregulated in expression to compensate for the perturbation. It is further respectfully submitted that amended Claim 35 as well as Claims 36 and former 37 (now claim 38) dependent thereto are novel in light of the teachings of Chattopadhyay *et al.* Amended claim 35 is now directed to a "*genetically modified yeast organism, comprising a genetically modified expression of at least one foreign gene, which results in compensating differential expression of at least one other gene endogenous to the modified yeast organism; and a phenotype caused by the reduction or elimination of the compensating differential expression of the gene, wherein the phenotype is perceptible from outside of the modified yeast organism and comprises behavior of the yeast organism, the morphology of the yeast organism, or a combination thereof.*"

As previously argued, Chattopadhyay *et al.* have no teachings that a genetically modified yeast organism is caused to express heterologously at least one protein or protein fragment by genetic modification by introducing a foreign gene into the yeast, wherein the expression does

not produce a detectable change in the phenotype perceptible from the outside of the yeast organism.

Thus, it is clear from amended Claim 35 that in a yeast organism of the instant Invention, a "foreign gene" is introduced into the yeast and is heterologously expressed to produce the at least one protein or protein fragment. Yet, Chattopadhyay *et al.* teach ***nothing*** with respect to yeast organisms having an introduced ***foreign gene*** that is heterologously expressed to produce at least one protein or protein fragment the encoded by the foreign gene. Quite the contrary, Chattopadhyay *et al.* clearly teach ***deleting endogenous genes*** from the yeast. Indeed, on page 6421, Chattopadhyay *et al.* clearly state:

> In this study, we demonstrate that ***deletion*** of either or both of the two genes HSP30 and BTN2, which have increased expression in *btn1*-Δ strains, did not alter the pH-dependent resistance to ANP in *btn1*-Δ strains nor did it result in resistance to ANP for *BTN1*$^+$ strains. Furthermore, the *btn1*-Δ *hsp2*-Δ *btn2*-Δ strain, with all three genes deleted, is viable and shows no growth defect under normal conditions (results not presented).
> (Emphasis added).

It is acknowledged that Chattopadhyay *et al.* inserted genes into the strains used in their experiments. However, there is no evidence these genes were expressed. Rather, Chattopadhyay *et al.* report these genes were inserted into endogenous genes to ***disrupt*** the expression of those endogenous genes. In the Materials and Methods section on page 6419, Chattopadhyay *et al.* make clear that:

> Deletion of *BTN1* has been described previously (22). Deletion of *HSP30* and *BTN2* was performed by standard techniques. *HSP30* was ***disrupted*** using the plasmid pSPHSP30 obtained from P. Piper

> (University College London). Briefly, a 1.1-kb *Hin*dIII fragment, containing the *URA3* gene inserted in the *Hin*dIII site in the coding region of *HSP30*, was used for ***disruption*** of *HSP30* by homologous recombination. *BTN2* was ***disrupted*** with the plasmid pAB2197 containing a 1.1-kb *Hin*dIII fragment with the *URA3* gene blunt ended and ligated in the *Nde*I site of the coding region of *BTN2*.

(Emphasis added.)

Thus, in Chattopadhyay *et al.*, the *URA3* gene was inserted, but no evidence was presented it was ever heterologously expressed.

MPEP § 706.02 specifically states that "...for anticipation under 35 U.S.C. 102, ***the reference must teach every aspect of the claimed invention*** either explicitly or impliedly. Any feature not directly taught must be inherently present (emphasis added)." Since Chattopadhyay *et al.* teach the ***deletion*** of endogenous genes and the insertion of a gene that does not undergo heterologous expression, ***it is impossible*** that Chattopadhyay *et al.* teach every aspect of the claimed Invention, either explicitly or impliedly. Hence, the teachings of Chattopadhyay *et al.* clearly ***do not*** anticipate amended Claim 35 and Claims dependent thereto.

35 U.S.C. § 103(a), Obviousness

Claims 24-31 and 34-37 have been newly rejected under 35 U.S.C. § 103(a) as being unpatentable over Srinivasan *et al.* (2000, Journal of Biological Chemistry, 275:29187-29192), Wilson *et al.* (1999, PNAS, USA 96:12833-12838), Sauer (1987, Molecular and Cellular Biology, 7:2087-2096) and Buchholz *et al.* (1998, Nature Biotechnology 16:657-662).

The Examiner has rejected claims 24-31 and 34-37 in view of Srinivasan *et al.*,

Wilson *et al.*, Sauer *et al.* and Buchholz *et al.* The Examiner alleges that an artisan would have wanted to study the effects of a second sod mutation in yeast because double-mutant yeast has a more severe phenotype than a single mutant. Thereby, a way to do so is outlined in Wilson *et al.* illustrating the concept of microarrays. Moreover, Sauer teaches the cre-lox recombination system for performing an inducible knock-out. Buchholz *et al.* teach looking for cre recombinases with better enzymatic properties.

Srinivasan *et al*, teach that a mutation in the SOD1 or 2 gene results in a lack of dismutase and thus an accumulation of oxygen radicals producing significantly elevated levels of EPR-detectable Fe (III). Lacking either the SOD1 or SOD2 gene shows different compromised phenotypes which sum up in double-mutant strains (see page 29187, right-hand column, first paragraph). The Examiner holds the view that an artisan would have wanted to study the effects of a second SOD mutation in yeast using the microarray of Wilson *et al.* and to introduce such a deletion mutation by making an inducible knock-out as taught by Sauer *et al.*

Applicants contend that combining Srinivasan *et al.* and Wilson *et al.*, Sauer and Buchholz *et al.* does not produce the present invention and thus the Examiner has not made a *prima facie* case of obviousness. In one embodiment of the present invention, a foreign gene is first introduced into a yeast organism and an altered phenotype is not apparent without analysis of the differentially expressed genes which compensate for the heterologously expressed gene. The phenotype as caused by introduction and expression of this foreign gene is suppressed (compensated) due to the modified expression of an endogenous gene whereby

the expression thereof has been modified as a reaction to the introduction of the foreign gene. Differently expressed genes are analyzed. In case of elevated expression, the expression is then reduced or eliminated resulting in a yeast strain with a phenotype reflecting the effects of the foreign gene. This stands in contrast to the teachings of Srinivasan *et al.* In Srinivasan *et al.*, a mutation has been introduced into an endogenous gene in contrast to the introduction of a foreign gene as comprised by the present invention. Furthermore, there is no indication in Srinivasan *et al.* that such mutation in an endogenous gene results in a modified expression of another gene which would compensate or suppress a phenotype affected by the first mutation. On the contrary, as stated on page 29187, right-hand column, 1st paragraph, yeast with a SOD1 mutation exhibits a specific phenotype which is different from that of the wild-type and is not compensated by a further endogenous mutation, namely poor growing in the air, sensitive to redox-cycling drugs, quick dying in stationary phase and lysine and methionine auxotrophy under aerobic conditions. A further mutation in the SOD2 gene resulting in a double mutant even worsens the condition of the yeast in that the phenotype as elicited by the SOD2 mutant sums up to the phenotype as caused by the SOD1 mutant. Thus, the SOD1-SOD2 mutated strain is in no way comparable to the strain as comprised by the present invention.

The Examiner has not made a *prima facie* case for obviousness of the claimed invention none of the other references remedies the situation. More specifically, methods known in the state of the art for studying effects on genes, such as for making an inducible knock-out or for looking for cre combinases with better enzymatic properties as disclosed by

Wilson *et al.*, Sauer *et al.* and Buchholz *et al.* cannot compensate for the defects of Srinivasan *et al.* as these merely suggest how to study effects or produce a knock out of a particular gene in a strain without suggesting the present invention, namely to introduce a foreign gene, then analyze the gene expression pattern and subsequently to reduce or eliminate expression of a gene which is upregulated in response to the introduction of the foreign gene and thus prevent an apparent phenotypic difference. In this respect, it should be kept in mind that introduction of a foreign gene in one embodiment of the present invention does not result in a detectable change of the phenotype due to the compensating expression of endogenous genes which stands in contrast to Srinivasan *et al.* wherein a SOD mutation results in a perceptible change of phenotype. The decisive matter distinguishing the present invention from Srinivasan *et al.* inter alia is that the present inventors work in a system in which modification of a gene is not perceptible and needs a further modification to render the effects of the modified first gene perceptible and have developed a method of how the "hidden" phenotype caused by a foreign gene can be rendered perceptible. This stands in contrast to Srinivasan *et al.* wherein the phenotype induced by a mutated gene and is clearly perceptible. Any methods known in the art for studying changes in gene expression cannot compensate for this deficiency in Srinivasan *et al.* and cannot render the present invention obvious over Srinivasan.

It is not clear how the Examiner arrives at the conclusion that a skilled person reading the document of Srinivasan *et al.* would have wanted to study the effect of a second SOD mutation. But even if the skilled person would do so, the cited references nevertheless do not

predict the present invention nor render it obvious. The present invention aims to prepare a yeast strain in which effects of heterologous gene expression are rendered perceptible by introducing further gene modifications to counter the effect of differentially regulated genes. In the yeast strain of Srinivasan *et al.*, the effects of a gene modification are perceptible *per se* without the need to apply further modifications on the genome. Moreover, Srinivasan *et al.* does not teach any compounds which are suitable to knock out the effects of the SOD2 gene in order for a skilled person to study the effects of such gene on the SOD1 gene. The purpose of Srinivasan *et al.* is to explore the iron status of yeast lacking SOD genes for monitoring the cellular oxidative status (see first section of discussion). No disclosure with respect to compensating the effects of a mutated gene is found in this reference.

It is also not clear how Buchholz et al. should compensate for the deficiency of Srinivasan *et al.* and suggest the present invention. The Examiner has not given a reason for her allegation that a skilled person would have screened for substances that affect activity of a recombinase. But even if the skilled person would do so, he/she would not arrive at the present invention, as the system used in Buchholz et al. is not a yeast cell in which the effects of a modified gene are perceptible only due to a modification of another gene. In both Srinivasan *et al.* and Buchholz *et al.*, modification of genes *per se* results in a perceptible phenotype without the need of modification of a compensating endogenous gene.

In view of the above, patentability should be acknowledged over Srinivasan *et al.*, Wilson *et al.*, Sauer *et al.* and Buchholz *et al.* and the rejection withdrawn.

Claims 24-26 and 30-37 have been newly rejected under 35 U.S.C. 103(a) as being unpatentable over DeRisi *et al.* (2000, FEBS Letters, 470:156-160), Gari *et al.*, (1997, Yeast 13:837-848), and Wilson *et al.* (1999, PNAS, USA 96:12833-12838).

The Examiner has rejected the claims over DeRisi *et al.*, Gari *et al.* and Wilson *et al.*, where DeRisi *et al.* allegedly discloses over expression of PDR1 and/or PDR3 in S. cerevisiae and identification of upregulated and down regulated genes. Moreover, the Examiner states that DeRisi *et al.* does not disclose a detectable phenotype as the morphology of the yeast is unaffected. Gari *et al.*, allegedly teach "tetracycline-regulatable promoter system, wherein tetracycline induces tetO-driven gene expression and induces expression of a gene of interest." Wilson *et al.*, allegedly teaches "changes in gene expression following treatment with a drug."

This rejection is respectfully traversed. DeRisi *et al.* teach strains which show a detectable phenotype which clearly constitutes a difference to the present invention. As stated on page 2, 1st paragraph of the present specification, 70%-80% of heterologously expressed kinases do not result in a detectable phenotype. Thus, the purpose of the present inventors was to generate an organism for drug screening by overcoming these disadvantages. The system applicants developed was an organism expressing a foreign gene which does not initially show a detectable change of phenotype and then inducing a change in genes responsible for masking any phenotypic change and thus revealing the altered phenotype. By the intentional introduction of a genetic alteration to such genes, the expression of which was modulated when introducing the foreign gene, the compensating

effect of the genes or gene products is eliminated and the "true" character of the foreign protein appears and is visible as change of phenotype.

The kind of phenotype that is changed and perceptible from the outside is thereby identified in claim 24 as being the behavior of the yeast organism, the morphology of the yeast organism or a combination thereof. There is no restriction that the phenotype is proliferation. The term "behavior" comprises a series of features that are perceptible from the outside. Thus, drug resistance as addressed in DeRisi *et al.* is included in this term as drug resistance results in the survival of a cell to which a drug is administered. This clearly falls within the term "behavior of the yeast organism" and is perceptible from outside. DeRisi *et al.* disclose a mutant yeast cell in which the pdr1, pdr3 genes are mutated. This results in a modified expression of a series of genes being either up or downregulated. The difference to present claim 24 (a), besides that in this item a foreign gene is introduced into the yeast organism, lies in the fact that the yeast organism of the present invention does not show a perceptible change of phenotype, *i.e.* the yeast remains unchanged with respect to behavior or morphology. In contrast thereto, the yeast cells of DeRisi *et al.* show a perceptible change of phenotype, namely the yeast cells enhance their drug resistance (see page 156, right-hand column, 1st and 2nd paragraph, and page 159, right-hand column, 3rd paragraph). This stands in clear contrast to the present invention wherein such a change of behavior is not perceptible. Thus, the genes which are differentially expressed have the effect of compensating genes as they suppress the actual phenotype caused by the foreign gene, whereas the differentially regulated genes in the yeasts of DeRisi *et al.* have no

compensating effect as drug resistance as conferred by Pdr1 and 3 is markedly enhanced despite the mutation of the genes. This is a change of the behavior of the yeast organism which is perceptible from the outside, thus constituting a difference to the present invention. Thus, the change of expression of genes induced by the pdr1, 3 mutations is not compensating.

As outlined above, one embodiment of the present invention is to generate yeast strains harboring a foreign gene. Unfortunately, the effect of the introduction of a foreign gene on phenotype, for a large number of foreign genes, is that no change in phenotype is perceptible due to compensation by endogenous genes. Such yeast would not be suitable for drug screening. Merely by the introduction of additional mutations to these compensating genes which are differently expressed upon introduction of a foreign gene, a genotype can be established which has a perceptible change with respect to the wild-type phenotype so that changes induced by drugs are more clearly visible. In contrast, the purpose of DeRisi *et al.* was to analyze transcriptional activation in multi-drug resistant yeast mutants and to identify differentially regulated genes in order to delineate the complex networks of genes involved in yeast pleiotropic drug resistance (page 156, right-hand column, fourth paragraph). Given this different purpose of DeRisi *et al.*, there is no reason to assume that a person skilled in the art would have turned back the changed expression of the up or downregulated genes as it was just the purpose of DeRisi *et al.* to identify genes which are differently expressed upon mutation of the pdr1 and 3 genes. Thus, it is irrelevant whether the prior art provides methods for knocking out upregulated genes or overexpressing downregulated genes as this

is not an purpose of DeRisi's work. There is no motivation in DeRisi *et al.* to change the expression of the differently expressed genes in order to investigate drug resistance. The recognition of the problem that heterologous expression of foreign genes often do not induce perceptible changes of phenotype and the solution of how this problem can be overcome, namely by changing expression of differently regulated genes, has only been proposed by the present inventors.

Moreover, with respect to the effects of a modification of expression of genes differentially expressed due to mutation in the pdr1/3 genes (see page 7, second paragraph and page 8, second paragraph of the Office Action), no clear statement can be given, as such subject matter is not examined in DeRisi *et al.* As shown by DeRisi *et al.* mutating the pdr1 and pdr3 genes results in a perceptible change of phenotype, one may speculate that the phenotype would be returned back to the original state, i.e. to a state as if pdr1 and pdr3 are not mutated, if the expression of up or downregulated endogenous genes would be reversed. Given the fact that in the yeast strain as used by DeRisi *et al.* the perceptible phenotype drug resistance is considerably increased, one may not assume that knocking out upregulated genes or overexpressing downregulated genes would eliminate yeast which is resistant to stress. Rather, one would assume that the drug resistance is turned back to normal. This, however, would result in a yeast organism showing no perceptible change of phenotype and being just the contrary of what is an embodiment of the present invention and thus would constitute teaching away from the present invention. Thus, just as the phenotype in the yeast organism of DeRisi *et al.* is changed in pdr1 and pdr3 mutants, the skilled person would not

have an incentive to eliminate a changed expression of endogenous genes and to turn it back to normal or even the opposite state as a normal drug resistance would not allow optimal screening for drugs as is one embodiment of the present invention.

One embodiment of the present invention is to generate a yeast organism showing the effects of an introduced foreign gene. The statement of the Examiner that the skilled person would have knocked out upregulated genes or overexpressed downregulated genes is based on mere hindsight giving the teachings of the present invention. Even if the skilled person performs such modifications to the system of DeRisi *et al.*, he/she would not arrive at the present invention because turning back the expression of differentially expressed genes would most likely turn back the phenotype to normal thus not constituting a yeast cell which is especially suitable for drug screening.

In contrast to DeRisi et al., in the present invention a foreign gene is introduced into the yeast organism and is expressed or overexpressed, however, in DeRisi *et al.*, a gene is mutated. Thus, the two systems are totally different: in the system of the present invention, the effect of a foreign gene on a cell is the subject, whereas in the system of DeRisi *et al.* the effect of a mutated endogenous gene on the cell is the subject. This is a further reason why DeRisi *et al.* is not relevant for the present invention. In view of this, the claims as presently amended are unobvious and are patentable over DeRisi *et al.*, Gari *et al.* and Wilson *et al.* and applicants request reconsideration and withdrawal of the rejection.

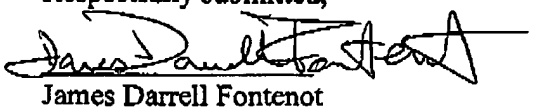
Fees

No fees are believed to be necessitated by the instant response. However, should this be in error, authorization is hereby given to charge Deposit Account no. 18-1982 for any underpayment, or to credit any overpayments.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks in the file history of the instant application. The claims as amended are believed to be in condition for allowance, and reconsideration and withdrawal of all of the outstanding rejections is therefore believed in order. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 908-231-2597.

Respectfully submitted,



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